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**CONTRACTING ORGANIZATION: University of Kentucky
Lexington, KY 40506**

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14. ABSTRACT Triple-negative breast cancer (TNBC) distinguishes from other forms of breast cancer in origination and progression. Likely originated from undifferentiated cancer stem cells, TNBC tumor cells possess many epithelial-mesenchymal transition (EMT) characteristics including invasion, resistance to apoptosis, and cancer stem cell-like traits that permit tumor dissemination and growth at distant sites. The Wnt pathways are important for EMT. We recently discovered that <i>Wnt5a</i> and its transcription factor Twist are markedly over-expressed in TNBC but not luminal breast cancer cells. We also discovered that constitutively activated NF- κ B in TNBC sustains prolonged activation of pro-inflammatory cytokines, enabling rapid spread (metastasis) of TNBC tumors. Notably, the functions of both transcription factors Twist and NF- κ B in gene activation require lysine acetylation, which signs to activate the transcriptional machinery in chromatin. This chemical modification enables them to recruit the major transcriptional regulatory co-activator proteins to coordinate target gene activation in the human genome. In this study, we will investigate the underlying mechanism of gene activation in TNBC. We are developing novel small molecule compounds to render the transcription factor/co-activator activity in gene activation, a key function required for the prolonged expression of inflammatory cytokines that fuel TNBC cells proliferation and spreading. Our study should have a major impact on new targeted therapy development to fight against the aggressive TNBC.						
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Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	2
3. Accomplishments.....	3
4. Impact.....	8
5. Changes/Problems.....	8
6. Products.....	8
7. Participants & Other Collaborating Organizations.....	9
8. Special Reporting Requirements.....	10
9. Appendices.....	10

1. Introduction

Breast cancer is the most commonly occupying cancer among women. While great stride is made in the recent years in disease diagnosis and treatment, we still don't have effective means to treat a major sub-population of metastatic breast cancer patients, particularly those who suffer from *triple-negative breast cancer* (TNBC). The average time to live after documentation of metastasis is only about two years. Unlike other subtypes, TNBC lacks the expression of three receptors: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu), hence the name. The available treatments targeting these receptors do not work for TNBC patients. Studies show that inflammatory genes drive rapid progression of TNBC, and epithelial-mesenchymal transition (EMT), a process of massive cell movement required for morphogenesis in embryonic development, is responsible for cancer cell invasion and metastasis. The major challenge in TNBC research is to identify the factors within the cell that initiate and promote tumor metastasis. Our goal is to determine the role of gene transcriptional regulation in the development of metastatic TNBC. We focus on the function of lysine acetylation in gene activation to attain both mechanistic insights and rational design of small molecules that modulate the acetyl-lysine binding activity of the bromodomain (BrD), which function was first discovered by the M.-M. Zhou lab (*Nature*, 1999) (PI of this project). BrDs are embedded in many transcription-associated proteins such as the BET (bromo and extra-terminal domain) proteins important for transcriptional activation of pro-inflammatory and oncogenesis genes in TNBC. Our recent study between the labs of Drs. M.-M. Zhou and B.P. Zhou (an expert investigator on TNBC; also Partnering PI of this project) has attributed rapid tumor growth and metastasis of TNBC cells to tumor initiating, undifferentiated stem cell properties, and to over-activation of oncogenes (*Cancer Cell*, 2014). We show that a new class of BrD inhibitors (BrDis), we developed, effectively down-regulate expression of cancer stem cell (CSC) factors, inhibit oncogenic gene expression, and suppress rapid growth and invasion properties of TNBC cells.

We hypothesize that the inflammatory, EMT and CSC properties of TNBC tumor are caused and fueled by transcriptional over-activation of inflammatory and oncogenic genes; chemical inhibition of such aberrant transcriptional activities can circumvent the aggression of metastatic TNBC tumor. To reach the goal of our proposed study, we will achieve the three Specific Aims: (1) Determine the role of BET proteins in gene transcriptional activation in TNBC; (2) Develop selective BrD inhibitors targeting oncogene-activation; and (3) Characterize the mechanism of the transcriptional program in TNBC cells.

EMT and CSC properties play a critical role in invasion, drug resistance, and tumor recurrence and are often associated with poor prognosis in TNBC patients. Our findings will contribute greatly toward the understanding of induction of EMT at metastasis. Our study also explores the therapeutic potential of targeting this initiating event for the treatment of metastatic breast cancer.

2. Keywords

BET – bromodomain and extra-terminal domain

BLBC – basal-like breast cancer

BrD – Bromodomain

ChIP – chromatin immunoprecipitation

ChIP-seq – chromatin immunoprecipitation sequencing

CSC – cancer stem cell

EMT – epithelial-mesenchymal transition

ER – estrogen receptor

FA – fluorescence anisotropy

FACS – flow cytometry analysis

Her2/neu – human epidermal growth factor receptor 2

ITC – isothermal titration calorimetry

NMR – nuclear magnetic resonance

PR – progesterone receptor

RNA-seq – RNA sequencing

TAMs – tumor-associated macrophages

TMA – tissue microarray

TNBC – triple-negative breast cancer

3. Accomplishments

3.1. What were the major goals of the project?

In this past 12 months of this project, i.e. the first year of this grant's funding period, we have focused our efforts in this study as outlined in the major Tasks 1-3 of our research proposal.

Task 1:

- a. Determine binding specificity of the BrDs of the BET proteins to lysine-acetylated peptides derived from histones and major transcription proteins including Twist, NF-kB and STAT3.
- b. Define the molecular basis of the BET BrDs' selective interactions with effector proteins through structure-guided analysis, and determining the key residues using site-directed mutagenesis.
- c. Validate the selective molecular interactions of the BET BrDs with transcription proteins in luminal and basal-like breast cancer cell lines, with and without treatment of new BET BrD inhibitors.

Task 2:

- a. Design and synthesize new diazobenzene analogs to optimize lead compounds with high affinity ($K_d < 100$ nM) and selectivity (>100:1 for a target over closely related proteins). This is an iterative process, and is coupled to **task 2.2b-c** and **task 3.1a-c**.
- b. Determine the detailed molecular basis of ligand recognition by the BET BrDs by obtaining SAR data of lead series, and by solving new crystal structures of new ligands bound to BET BrDs.
- c. Validate the cellular efficacy ($EC_{50} < 1 \mu M$) of new BrD inhibitors in multiple TBNC cell lines.

Task 3:

- a. Elucidate BRD4 functions in EMT and CSC properties as well as tumorigenicity of TNBC cells *in vitro* and *in vivo* using the newly developed selective BrD inhibitors.
- b. Identify direct target genes of BRD4 in TNBC cell lines through ChIP-seq and RNA-seq analysis.
- c. Determine the transcriptional expression levels of target genes of BRD4 in human TNBC samples.

3.2. What was accomplished under these goals?

We have made major progress in the past 12 months in this collaborative study between Dr. M.-M. Zhou's Lab at Icahn School of Medicine at Mount Sinai and Dr. B.H. Zhou's Lab at Kentucky University College of Medicine, as planned in our proposal. Specifically, Dr. M.-M. Zhou's lab has conducted extensive structural and biochemical analyses of interactions of the bromodomains of BET proteins, particularly BRD4 with lysine-acetylated histones and transcription factors including Twist, NF-kb, STAT3, and FOXO3a, and conducted structure-based rational design and synthesis of new chemical inhibitors for BET BrDs. At the same, Dr. B.H. Zhou's lab has performed detailed functional characterization of BET proteins interactions with histones and key transcriptional factors using the new structural insights generated from Dr. M.-M. Zhou lab's study. In addition, Dr. B.H. Zhou's lab has also evaluated the new BrD inhibitors, and used them to discover new molecular mechanism underlying the role of FOXO3a/BRD4 interaction in drug resistance in breast cancer (see below). In doing so, we have completed **subtasks 1a** and **1b**, and major portion of **subtasks 2a** and **2b** (M.-M. Zhou), and **subtasks 1c** and **3c** (B.H. Zhou), and **subtasks 2c** and **3a** (M.-M. Zhou and B.H. Zhou). Below, we highlight some of our new key discoveries in this joint project.

(A) Structural Mechanism of FOXO3a/BRD4 in Control of Gene Transcription

The BET proteins, BRD4, BRD3, BRD2, and testis-specific BRDT, are characteristic of two BrDs followed by the ET domain and function to regulate gene transcription in chromatin. Specifically, BRD4 of the BET family recruits transcription factors to target gene enhancer and promoter sites, promotes assembly of *cis*-regulatory enhancer elements, and activates transcriptional elongation by RNA polymerase II for productive gene transcription, all of which relies on the acetyl-lysine binding activity of its BrDs (**Figure 1A**). Growing evidence including our own study shows that inhibitors of BET BrDs, i.e. blocking their binding to lysine-acetylated histones and transcription proteins, have emerged as promising therapeutics in cancer through block BRD4 binding to histones and transcription factors, however drug resistance has also encountered

warranting better understanding of mechanisms of BRD functions. Notably, our recent studies show that numerous transcription factors rely on lysine acetylation to recruit BRD4 for target gene activation. Specifically, we discovered that the two BrDs of BRD4 together with a di-acetylation motif in histone H4 and a transcription factor (TF, e.g. Twist) cooperatively form a ternary complex of TF/BRD4/H4 at distinct promoter site wherein the 2nd BrD (BD2) binds to di-acetylated TF and the 1st BrD (BD1) binds to di-acetylated H4 (**Figure 1A**). Our structure-guided sequence analysis further detects this di-acetylation motif in the FOXO family of transcription factors (**Figure 1B**). NMR structural analysis further revealed that a FOXO3 K242ac/K245ac peptide binds preferentially to the BD2, but much less to the BD1 of BRD4 in a dose-dependent manner (**Figure 1C**). We further confirmed that FOXO3 interacts with the BD2 but not BD1 of BRD4 when exogenously expressed in HEK293T cells in an acetylation-dependent manner and sensitive to BET BrD inhibition by our chemical inhibitor, MS417 (**Figure 1D**). FOXO3 interactions BRD4 BD2 is nearly lost when K242/K245 are mutated to Arg, as shown in HEK293T cells (**Figure 1E**). Finally, we detected that endogenous BRD4 interacts with FOXO3a in basal-like breast cancer cells, T47D, which is inhibited by MS417 or JQ1 treatment (**Figure 1F**). Collectively, our new insights of the structural mechanism of FOXO3a/BRD4 interaction laid an important foundation to elucidate its functional importance in the PI3K/AKT-FOXO3a/BRD4-CDK6 axis in the drug resistant breast cancer cells due to extended use of PI3K/AKT kinase inhibitors (see below).

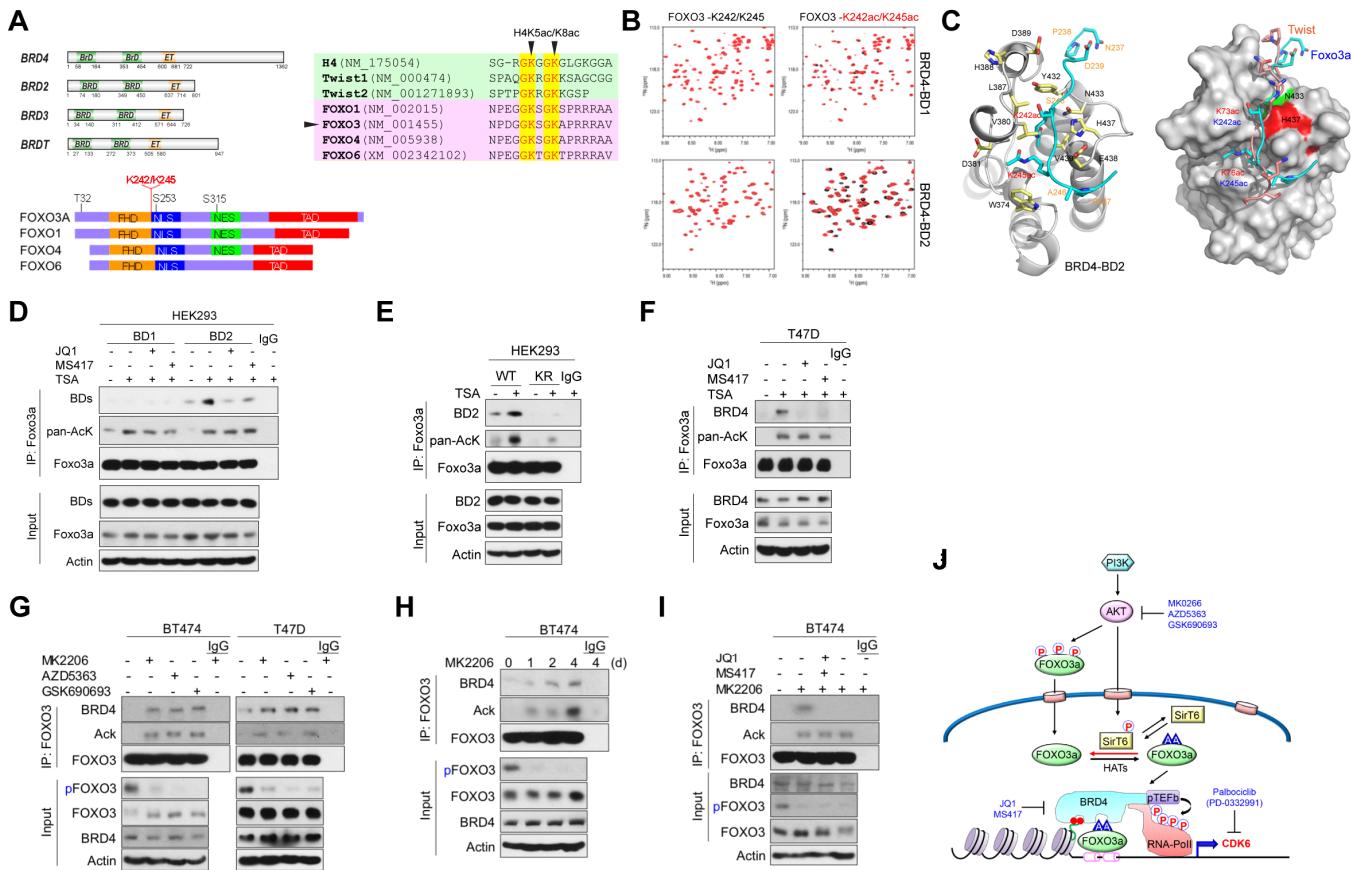


Figure 1. FOXO3/BRD4 association is mediated by K242ac/K245ac in FOXO3. (A) Upper, domain organization of BET proteins. Lower, sequence alignment of di-acetylation sites in H4, Twist and FOXO family TFs; (B) 2D ^{15}N -HSQC spectra showing that BRD4-BD2, but not BD1 exhibits NMR resonance changes upon addition of a FOXO3-K241ac/K244ac peptide (red vs. black); (C) NMR 3D structure of BRD4-BD2 bound to FOXO3-K242ac/K245ac peptide (cyan); (D) IP of Foxo3a from HEK293T cells, transfected with HA-tagged BRD4 BD1 or BD2; Right, IP detecting Foxo3a binding to endogenous BRD4 in HEK293T cells; (E) FOXO3a/BRD4-BD2 interaction was lost when K242/K245 in FOXO3a were mutated in Arg, as shown in HEK293T cells; (F) Effects of FOXO3a/BRD4 inhibition by MS417 or JQ1 in T47D BLBC cells. (G) Treatment of AKT inhibitors (MK2206, AZD5363 and GSK690693) resulted in enhanced association of FOXO3a/BRD4 in breast cancer cell lines BT474 and T47D; (H) Drug resistance due to extended use of AKT inhibitor, MK2206 involves an increased association of FOXO3a/BRD4 as shown in BT474 cells; (I) Combinatorial treatment of AKT inhibitor and BET inhibitor, MS417 or JQ1 overcomes drug resistance through blocking FOXO3a/BRD4 interaction; (J) Schematic diagram illustrating the PI3K/AKT-FOXO3a/BRD4-CDK6 axis that represents as a major mechanism underlying drug resistance induced by an extended use of PI3K or AKT inhibitors in breast cancer.

B. FOXO3a/BRD4 Binding Constitutes a Key Step in AKTi Induced Drug Resistance in Breast Cancer

Despite their initial promising as a targeted anti-cancer therapy, clinical development of small molecule kinase inhibitors targeting the PI3K/Akt signaling pathway has encountered serious setback due to drug resistance of tumor cells. Recently, we found that treatment with three different AKT inhibitors (AKTis) significantly induced FOXO3a acetylation and BRD4 association, concomitant with the suppression of FOXO3a phosphorylation (**Figure 1G**). The level of FOXO3a acetylation and its interaction with BRD4 induced by AKTi are in a time-dependent manner, it appeared at day 1 and reached to maximum at day 4 after MK2206 treatment (**Figure 1H**), suggesting that the FOXO3a-BRD4 interaction is a late-stage event and involves in drug resistance. The FOXO3a-BRD4 interaction is sensitive to BRD4 inhibition, because addition of JQ1 or MS417 in the immunoprecipitation (IP) buffer completely disrupted the FOXO3a-BRD4 interaction in BT474 cells (**Figure 1I**). The latter was further confirmed by endogenous FOXO3a/BRD4 interaction in T47D cells. Collectively, these data indicate that AKTi treatment induces the acetylation of FOXO3a and its interaction with BRD4, which can be disrupted by JQ1 or MS417.

In the last several months, we have been examining therapeutic efficacy of our new BrD inhibitors along with JQ1 (as control) in the suppression of proliferation, migration and invasion of TNBC cell lines. In addition, we treated these cell lines with BRD4 inhibitors in combination with various therapeutic agents. Surprisingly, we discovered that BRD4 inhibitors synergize the growth suppressive effect of AKTis. These results suggested that BRD4 inhibition can increase the growth suppressive effect mediated by AKTi (MK2206, AZD5363 and GSK690693). This effect seems to be specific, because we used three different AKTi with different mechanistic action on AKT inhibition. For example, MK2206 suppresses the allosteric activation of AKT whereas AZD5363 binds to the catalytic pocket of AKT. To further determine that the synergistic effect observed in the combination of BRD4 inhibitor with three different AKTi is not likely due to off-target effect, we also knocked down the expression BET family members BRD4, BRD3, BRD2 and BRDT individually and treated these cells with AKTi, we found that only BRD4-knockdown greatly enhanced the suppressive effect of AKTi. These results suggested that BRD4 inhibition can increase the growth suppressive effect mediated by AKTi.

In line with these new findings, we performed cDNA microarray analysis to identify the transcriptional target induced by BRD4 inhibitors and AKTi. Among the 40 overlapping genes, CDK6 is noted as an oncogenic kinase that governs G1/S phase transition and cell cycle progression. Consistent with the microarray data, AKTi treatment induced the protein and mRNA levels of CDK6 in various breast cancer cell lines. The induction of CDK6 by AKTi was time dependent, which starts at day 1 and reach to maximal at day 4 in these cell lines. The pattern of CDK6 induction by AKTi is similar to the increased FOXO3a acetylation and its interaction with BRD4 mediated by AKTi, suggesting that CDK6 expression is regulated by the FOXO3a-BRD4 complex. In line with CDK6 induction, E2F-promoter driven luciferase was upregulated in cells treated with AKTi. Intriguingly, treatment with JQ1 or MS417 abolished AKTi-mediated E2F-promoter luciferase activity, suggesting that BRD4 inhibitor can suppress in the induction of CDK6. Indeed, treatment with JQ1 or MS417 blocked MK2066-mediated CDK6 induction in breast cancer cells. These results suggest that CDK6 induction mediated by AKTi is a general phenomenon and is likely mediated by the FOXO3a-BRD4 complex, illustrated in a schematic diagram shown in **Figure 1J**. These important and exciting findings were currently under further investigation and a new manuscript will be prepared for submission soon.

C. Structure-Guided Design of New BrD Inhibitors for the BET Proteins

We aim to develop potent and selective compound inhibitors that target the first bromodomain (BD1) of BRD4, which plays an important role in sustaining transcriptional over-activation of oncogenes required for TNBC tumor growth and metastasis, as demonstrated in our recent study (*Cancer Cell*, 2014). Guided with our unique structural insights of BRD4 BrD/ligand recognition, we have completed two rounds of design, synthesis and structure-activity relationship (SAR) characterization of lead optimization. Specifically, we have synthesized and evaluated about 25 new diazobenzene-based BrD inhibitors. Of these, we have obtained a new inhibitor, MS611 that has very promising 100-fold selectivity for the BD1 of BRD4 over the BD2. Our new crystal structural analysis of MS611 bound to BRD4-BD1 reveals that our newly designed cyano-phenyl motif, positioned *para*- to the sulfonamide connectivity, establishes direct interactions with two key residues Lys91 and Asp145 that are unique for BD1, thus explaining MS611's superior selectivity

(Figure 2A-B). We further observed that MS611 indeed has much better beneficial effects than pan-BET BrD inhibitors such as MS417 or JQ1 in modulating gene transcription in biological processes. Furthermore, in an effort to develop highly selective BrD inhibitors to BRD4, we have also explored a new idea of targeting two BrDs of BRD4 simultaneously together with one chemical inhibitor. This is achieved by chemical linking two BrD inhibitors (monovalent BrDis) to generate a bivalent BrDi. Indeed, we demonstrate that our newly synthesized bivalent BrDi, MS645 is much more potent than monovalent BrDi MS417 in inhibiting rapid cancer cell growth with a panel of TNBC cell lines tested (Figure 2C). We have initiated a set of biological experiments to functionally evaluate such new bivalent BrDis in *in vivo* mouse xenograft model study.

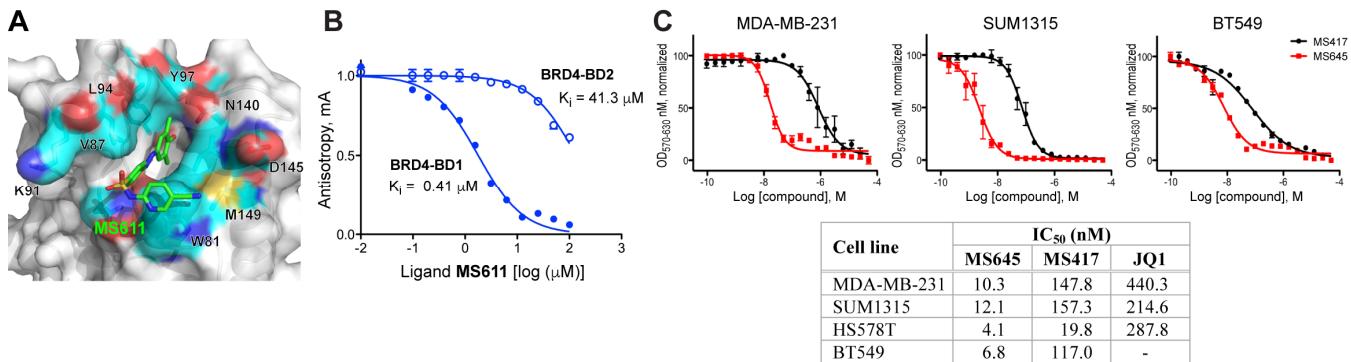


Figure 2. Structure-based design of selective BrDis. (A) Crystal structures of BRD4-BD1 bound to a lead BrDi MS611 (green); (B) Selective binding of MS611 for BRD4 BD1 over BD2, as measured by using a fluorescence anisotropy assay with a FITC-labeled MS417 as an assay probe; (C) Study of TNBC cell growth inhibition by bivalent BrDi MS645 vs. monovalent BrDi MS417, as measured by MTT assay.

3.3. What opportunities for training and professional development has the project provided?

In the last funding period, the professional development of both Drs. Ming-Ming Zhou and Binhu P. Zhou was further strengthened and broaden as indicated in their active participation of breast cancer-related grant review at the NCI, DoD and Komen Cancer Foundation, as well as professional activities in reviewing scientific journals, and the presentation at several meetings and institutes, as shown below:

A. Grant Review:

Ming-Ming Zhou

2014 - Regular Member, NIH - “Macromolecular Structure and Function B” (MSFB)

Binhu P. Zhou

10/2015 Become standing member, TPM study section, National Cancer Institute (NCI)

06/2015 Reviewer, DOD BRCP Breakthrough Award Panel (PBY3)

05/2015 Reviewer, Mary Kay Ash Foundation for Cancer Research Grants, Dallas, TX

01/2016 Reviewer, Susan G. Komen Foundation

B. Editor/Service on Editorial Boards:

Ming-Ming Zhou

2009 - Editorial Board, *Journal of Molecular Cell Biology*

2010 - Editorial Board, *ACS Medicinal Chemistry Letters*

2010 - *Faculty of 1000* on “Structure, and Transcription and Translation”

2012 - Editorial Board, *Journal of Cancer Immunology*

2015 Editorial Board, *Molecular Cancer Therapeutics* (ACS)

2015 Editorial Board, *World Journal of Biological Chemistry*

2015 Co-Organizer, 2015 FASEB Research Conference on “HDACs, Sirtuins and Reversible Acetylation in Signaling and Disease”, (Co-Organizer, David Sinclair)

Binhu P. Zhou

2015 - Associate Editor, *Molecular and Cellular Oncology*

2015 - Consulting Editors, *JCI Insight*

2015 - Editorial board member: *Scientific Reports*,
 2015 Guest Editor, Special issue of "Epithelial-mesenchymal Transition in Cancer Progression and Metastasis", *Cancer and Metastasis Reviews*

3.4. How were the results disseminated to communities of interest?

We have been disseminating the results of our study to the research community through invited talks at the universities and scientific conferences in the past 12 months, as well as publications:

A. Presentations

Ming-Ming Zhou

03/2015 University of Iowa, Department of Biochemistry, Iowa City, Iowa
 03/2015 Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN
 04/2015 The Children's Hospital of Philadelphia, The University of Pennsylvania School of Medicine, Philadelphia, PA
 05/2015 University of California San Francisco, Pharmacology and Pharmacogenomics Program, San Francisco, CA
 08/2015 2015 FASEB Research Conference on "HDACs, Sirtuins and Reversible Acetylation in Signaling and Disease", Co-Organizer, Germany (with D. Sinclair)
 12/2015 PacifiChem 2015, Symposium on "Frontiers in Chromatin Biology and Chemical Epigenetics/Epigenomics," Honolulu, Hawaii
 03/2016 New York Genome Center, NY Cancer Genomics Research Network, NY
 03/2016 2016 ACS National Meeting, Symposium on "Bromodomain Inhibition: BETs and Beyond", San Diego, CA
 04/2016 University of Florida College of Medicine, Center for Epigenetics, Gainesville, FL

Binhu P. Zhou

03/2015 Department of Biochemistry, University of Florida, Gainesville, FL
 03/2015 Elkin lecture, Winship Cancer Center, Emory University School of Medicine, Atlanta, GA
 04/2015 Department of System Biology, University of Pittsburgh, Pittsburgh, PA
 05/2015 Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC
 05/2015 Karmanos Cancer Institute, Detroit, Michigan
 08/2015 Cancer Biology Program, City of Hope, Los Angeles, CA
 08/2015 Houston Methodist Research Institute/Weill Medical College at Cornell University, Houston, TX
 11/2015 Stephenson Cancer Center, University of Oklahoma Health Science Center, Oklahoma City, OK
 12/2015 Breast Cancer Research Program, University of California, Los Angeles, CA
 12/2015 Department of Medicine & Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

B. Publications relevant to this project

Hu, Y., Zhou, J., Ye, F., Xiong, H., Peng, L., Zheng, Z., Xu, F., Cui, M., Wei, C., Wang, X., Wang, Z., Zhu, H., Lee, P., **Zhou, M.-M.**, Jiang, B., & Zhang, D.Y. (2015) BRD4 Inhibition Inhibits Tumor Growth and Metastasis in Colorectal Cancer. *International Journal of Molecular Sciences*, 16(1): 1928-48. 2015 Jan 16. PMID: 25603177. PMCID: PMC4307342.

Smith, S.G., & **Zhou, M.-M.** (2015) The Bromodomain as the Acetyl-Lysine Binding Domain in Gene Transcription in "Histone Recognition" (M.-M. Zhou, Ed.) Springer (DE), Heidelberg, Germany, pages 1-26.

Stratikopoulos, E.E., Dendy, M., Szabolcs, M., Khaykin, A.J., Lefebvre, C., **Zhou, M.-M.**, & Parsons, R. (2015) Kinase and BET Inhibitors Together Clamp Inhibition of PI3K Signaling and Overcome Resistance to Therapy. *Cancer Cell*, 27, 837-851. PMID: 26058079. PMCID: PMC.

Sharma, R., & **Zhou, M.-M.** (2015) Partners in Crime: The Effects of Tandem Modules in Gene Transcription. *Protein Sciences*, 24(9):1347-1359. 2015 Sept. Epub 2015 Jun. 11. PMID: 26059070.

Zhang, G., Smith, S.G., & **Zhou, M.-M.** (2015) Discovery of Chemical Inhibitors of Human Bromodomains. *Chemical Reviews*. 115(21):11625-68. Epub 2015 Oct 23. PMID: 26492937.

Smith, S.G., & Zhou, M.-M. (2015) The Bromodomain: A New Target in Emerging Epigenetic Medicine. *ACS Chem. Biol.* Epub 2015 Nov 23. PMID: 26596782.

3.5. What do you plan to do during the next reporting period to accomplish the goals?

We are continuing our efforts in dissecting the molecular mechanism of gene transcriptional regulation or mis-regulation underlying metastatic breast cancer. Given that the major questions remained are on how lysine acetylation-mediated protein-protein interactions work in combinatorial fashions in gene transcription in chromatin, our ongoing efforts focus on the structural mechanism-guided rationally designed functional study in order to obtain deep functional and mechanistic underpinning of different transcription factors and regulatory proteins in their functions in control of gene transcription in the disease state and in response to pharmacological inhibition. *In summary*, we have made major progress in the past 12 months (i.e. the first year of this grant) towards addressing most aspects of the proposed studies as stated in the major tasks in the Statement of Work. We are continuing our efforts to achieve the goals of the remaining aims on the investigation of new therapeutic strategy that targets mis-regulation of oncogene transcriptional activation in metastatic breast cancer.

4. Impact

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer that is associated with early metastasis to brain and lung, poor prognosis and short survival. About 240,000 women were diagnosed worldwide in 2012 with breast cancer, of which ~20-25% are of TNBC. TNBC disproportionately affects women of African and Hispanic descent, and occurs more often in younger women, affecting women as early as in their 20s. 80% of breast cancer in people with an inherited BRCA1 mutation is found to be TNBC. TNBC lacks expression of three receptors, i.e. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu), hence its name. Most available breast cancer treatments target these receptors. Unfortunately, given their triple negative status, TNBC tumors generally do not respond to receptor-targeted treatments. Depending on the stage of its diagnosis, TNBC is very aggressive, highly metastatic, and much more likely to recur than other breast cancer subtypes. Currently, there is no targeted therapy for TNBC. The standard of care for TNBC is surgery with adjuvant chemotherapy and radiation therapy, which is not effective once the tumor is spread.

Recent studies suggest that TNBC is inflammation-associated cancer - its rapid tumor growth and metastasis is heavily dependent upon and fueled by markedly elevated transcriptional activation of pro-inflammatory cytokines and EMT program. As such, chemical inhibitors that target epigenetic proteins whose functions are required for over-expression of these oncogenes offer an exciting opportunity to develop a new targeted epigenetic therapy to fight triple-negative breast cancer. Therefore, the funding provided by the DoD Breast Cancer Breakthrough Award will greatly accelerate our ongoing efforts to test our hypothesis, and validate our novel lead chemical compounds as a potentially new targeted epigenetic therapy to fight against this aggressive and devastating disease.

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	Ming-Ming Zhou
Project role:	PI
Researcher identifier:	#1 at ISMMS
Nearest person months worked:	1
Contribution to project:	Directing the project
Funding sources:	
Name:	Adam Vincek
Project role:	Chemist
Researcher identifier:	#2 at ISMMS
Nearest person months worked:	6
Contribution to project:	Synthesis of new chemical compounds for BrDs
Funding sources:	
Name:	Guangtao Zhang
Project role:	Biochemist
Researcher identifier:	#3 at ISMMS
Nearest person months worked:	6
Contribution to project:	SAR study of BET BrD/ligand binding
Funding sources:	
Name:	Jamel Meslamani
Project role:	Structural Chemist
Researcher identifier:	#4 at ISMMS
Nearest person months worked:	2
Contribution to project:	Structure-based design and analysis of BRD4 BrDs
Name:	Binhua P. Zhou
Project role:	PI
Researcher identifier:	#1 UKSoM
Nearest person months worked:	1
Contribution to project:	Directing the project
Name:	Yuting Zhou
Project role:	Graduate Student
Researcher identifier:	#2 UKSoM
Nearest person months worked:	4
Contribution to project:	Conducted identification of the interaction of BDR4 with FOXO3a and characterization of the target gene CDK6 of the BRD4-FOXO3a complex.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

In the past 12 months, Dr. Ming-Ming Zhou has some changes in his group's research grants, as listed below:

New Research Support

1R01AI124465-01 (PI: M.-M. Zhou) 04/01/2016 – 03/31/2021 2.0 cal. mon.
NIH/NIAID \$250,000/yr, d.c.
"Mechanism of BET Proteins in Th17 Cell Differentiation"
This project aims to investigate the gene transcriptional program involving BET proteins during Th17 cell development.

Completed Research Support

R01HG004508-05 (PI: Zhou) 02/15/2012 – 01/31/2015 2.5 cal. mon.
NIH/NHRGI \$250,000/yr, d.c. (Zhou lab)
"Chemical Genomics Paradigm for Epigenetics Regulation"
The Specific Aims of this project are to: (1) develop new methodologies for genome-wide structural and biochemical analysis of protein-protein interactions in histone biology; and (2) design chemical tools to study biological functions of proteins involved in epigenetic gene transcriptional regulation.

R33DA029963-03 (MPIs: M. Ohlmeier, & M.-M. Zhou) 08/01/2010 – 07/31/2015 0.6 cal. mon.
NIH/NIDA \$115,000/yr, d.c. (Zhou Lab)
"Small Molecule Libraries Targeted to CBP and Attenuation ΔfosB Expression"
The Specific Aims of this project are to: (1) design and synthesize small molecules that inhibit CBP transcriptional activity; (2) structural analysis of target recognition by the CBP inhibitors; and (3) evaluate the new chemical ligands that inhibit CBP function in transcriptional activation of *ΔfosB*.

1R01CA154809-02 (MPIs: M. Walsh; & M.-M. Zhou) 01/01/2011 – 12/31/2015 1.0 cal. mon.
NIH/NCI \$142,500/yr, d.c. (Zhou Lab)
"Non-coding RNAs for epigenetic transcriptional silencing in prostate cancer"
The Specific Aims of this project are to: (1) investigate the molecular basis of H3K27me3 and non-RNA interactions with Polycomb repressive complex proteins; and (2) investigate the mechanistic role of long non-coding RNAs in epigenetic control of transcriptional silencing of *Hox* genes in prostate cancer.

8. Special Reporting Requirements

N/A

9. Appendices

N/A